

Nutrient Supply Rate and Mycorrhizal Colonization Control Patterns of Element Distribution in Ectomycorrhizal Pine

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Abstract: Ectomycorrhizal fungi may provide plants access to nonexchangeable nutrients. We measured nutrients (potassium, calcium, magnesium, manganese, iron, and aluminum) in roots and foliage in nonmycorrhizal and ectomycorrhizal *Pinus sylvestris* cultured in perlite at two nutrient supply levels. We also measured nutrients in perlite leachates from abiotic experiments using hydrochloric or oxalic acid at pH 2–4. Twenty-one percent more potassium and 30% more calcium accumulated in nonmycorrhizal plants than in ectomycorrhizal plants, presumably because of nutrient sequestration in extraradical fungal biomass. Plants at low nutrient supply accumulated 22% more potassium and 23% more calcium than at high nutrient supply, presumably because of additional mobilization of nutrients from perlite by plant and fungal acids. Significantly more leaching at pH 2 with oxalic than with hydrochloric acid occurred, probably caused by enhanced ligand-mediated dissolution with oxalic acid. Leaching of minerals by organic acids may enhance plant nutrient supply, particularly from microsites of low pH.

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INTRODUCTION

The ectomycorrhizal fungi found in symbiotic relationships with pines largely mediate nutrient uptake. Most work in the field and laboratory has focused on how these fungi supply nitrogen (N) and phosphorus (P) (Read and Perez-Moreno 2003), but some field studies have shown that these fungi can also supply base cations and trace elements (e.g., Wallander and Wickman 1999). Increased leaching of these nutrients by anthropogenically derived acid rain may cause these nutrients to limit plant growth (Wallander 2006). However, few studies have examined (under controlled conditions) how ectomycorrhizal fungi influence plant nutrient supply of base cations.

Nutrient supply in plants is generally considered to involve uptake of nutrients from the soil solution or from cation exchange sites on clays or organic matter (Mengel and Kirkby 2001). However, evidence is mounting that ectomycorrhizal fungi can provide plants access to nutrients stored either in soil humic material (Bending and Read 1995) or in minerals themselves (Paris et al. 1995; Paris, Botton, and Lapeyrie 1996; Wallander and Wickman 1999; Van Breemen et al. 2000).

In some tree species, the secretion of organic acids such as citrate or oxalate by ectomycorrhizal fungi can facilitate nutrient mobilization and increase concentrations of oxalate and citrate in the rooting zone (Wallander, Wickman, and Jacks 1997). Complexation with organic acids, particularly oxalate, increases the solubility of many elements, including aluminum (Al), iron (Fe), calcium (Ca), and magnesium (Mg) (Ahonen-Jonnarth et al. 2000; Cumming et al. 2001), potentially making them available for plant and fungal uptake. Oxalic acid can also increase dramatically rates of mineral dissolution (Stillings et al. 1996).

Nutrient availability in trees is primarily assessed by foliar analysis of nutrient concentrations (Knecht and Göransson 2004). Yet, unequal distribution of nutrients among different tissues (such as between foliage and root tissues) of a tree may indicate that nutrient availability assessed through the foliage differs from whole-tree nutrient availability. The distribution of nutrients among tree components is difficult to examine in field studies, but culture studies may provide valuable information on how nutrients are partitioned between major tissues.

One powerful technique to examine plant nutrient dynamics in culture is to supply plants with nutrients at exponentially increasing rates (Ingestad and Lund 1986). Under these conditions, plant growth rates adjust to reflect the rate of nutrient addition, and plants thereby achieve

stable internal nutrient concentrations. These techniques have subsequently been adapted to culturing symbioses of pine seedlings and various ectomycorrhizal fungi under semihydroponic conditions (Ingestad, Arveby, and Kähr 1986; Colpaert, Van Laere, and Van Assche 1996). Although such studies have allowed C, N, and P dynamics in ectomycorrhizal systems to be examined at different rates of nutrient supply and with different ectomycorrhizal fungi (Colpaert et al. 1999; Hobbie and Colpaert 2003, 2004), this approach has not been widely applied to examine nutrient concentrations of other elements.

In this study, we examined nutrient concentrations [potassium (K), manganese (Mn), Ca, Mg, Fe, and Al] in foliage and roots of the important timber species Scots pine (*Pinus sylvestris* L.) in seedlings grown in perlite at two different rates of nutrient supply (3% day⁻¹ and 5% day⁻¹). To examine the influence of colonization by ectomycorrhizal fungi on plant nutrients, the seedlings were also cultured with two fungal symbionts that differed in their organic acid production. Because perlite could be a potential source of nutrients (Enkelmann 1992), we measured nutrient leaching from perlite by hydrochloric acid or oxalic acid at pH 2, 3, and 4. Our primary interests in the study were to examine treatment effects on element concentrations in foliage and roots and examine whether perlite could be a potential nutrient source. We also combined results of the leaching experiments with elemental mass balance calculations to assist in quantifying sources of the elements.

MATERIALS AND METHODS

In this experiment, we used samples that had been previously investigated for C and N dynamics (Hobbie and Colpaert 2003, 2004).

Culture Conditions

Surface-sterilized *Pinus sylvestris* seeds were initially sown in a perlite/vermiculite (2/1, v/v) mixture moistened with a balanced nutrient solution for *Pinus sylvestris* (Ingestad and Kähr 1985). The stock solution contents are given in μM as follows: potassium sulfate (K_2SO_4 ; 56), potassium nitrate (KNO_3 ; 77), monopotassium phosphate (KH_2PO_4 ; 50), dipotassium phosphate (K_2HPO_4 ; 46), ammonium nitrate (NH_4NO_3 ; 585), calcium nitrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 29], magnesium nitrate [$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; 49], boric acid (H_3BO_3 ; 4), manganese nitrate [$\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 1.5], iron nitrate [$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$; 2.5], zinc nitrate [$\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 0.1], copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; 0.1), and sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 0.01). The pH was adjusted to 4.5 by adding

hydrochloric acid. Nitrogen, the growth-limiting element, was supplied by 41% ammonium and 59% nitrate. The experiment was carried out in a growth chamber with $300 \text{ mol m}^{-2} \text{ s}^{-1}$ photosynthetically active radiation, at least 70% relative air humidity, and a day/night rhythm of 18/6 h and 22/15 °C. Twenty-eight days after sowing, 35 uniform seedlings were selected for the experiment. A sandwich technique was used to inoculate 20 seedlings (Van Tichelen and Colpaert 2000) with either *Suillus luteus* L. or *Thelephora terrestris* Ehrh. In this technique, mycorrhizal plugs were grown in a plastic Petri dish containing modified Melin–Norkrans agar medium covered with sterile cellophane sheets. Once mycelia had covered most of the cellophane, the agar was replaced with thick filter paper (9 cm in diameter) soaked in Ingestad nutrient solution. For each Petri dish, root systems of two seedlings were spread over the young mycelia, with stems placed in a notch of the side wall of the Petri dish. A second filter paper was used to cover the roots and mycelia. Roots remained in contact with the mycelia for 3 days before transfer to 70-cm³ containers filled with 4.6 g acid-washed, sieved perlite (2 to 4-mm particles) (Colpaert et al. 1999), a mineral of low cation exchange capacity. Ten nonmycorrhizal seedlings followed the same procedure in the absence of fungal inoculum, of which five were immediately harvested to determine biomass and total plant nutrients at inoculation.

Perlite is an expandable hydrated siliceous volcanic glass of rhyolite composition with an average of 70–77.5% of silicon dioxide (SiO_2), 12–15% aluminum oxide (Al_2O_3), 3–5% potassium oxide (K_2O), 0.5–2% iron oxide (Fe_2O_3), 0.5–1.5% calcium oxide (CaO), 0.2–0.7% magnesium oxide (MgO), 3–5% H_2O , and traces of other elements. Perlite has a low nutrient buffering capacity, so plants grow in a semihydroponic environment where nutrient addition and nutrient uptake in the plants are assumed to be equivalent. Perlite is highly susceptible to both physical and chemical weathering because of a high fracture density.

Immediately after inoculation, two different nutrient supply rates were applied. Nutrients were either added at a constant relative addition rate of 3% day⁻¹ (low nutrient treatment) or 5% day⁻¹ (high nutrient treatment). These nutrient regimes are suboptimal so that seedlings will adjust their relative growth rate to nutrient addition rates (Ingestad and Kähr 1985). Nutrient weight proportions supplied to the plants in both treatments were identical [100 N–15 P–67 K–6 Ca–6 Mg–19 sulfur (S)].

Culture Harvesting and Biomass Measurements

Plants were harvested once the cumulative N added to each plant during the experimental period was 8.0 mg, after either 45 days (high N

treatment) or 70 days (low N treatment). Plant shoots were cut. Nonabsorbed N was washed from the perlite with 200 ml of N-free nutrient solution. Subsequently, containers were centrifuged at 135 g for 30 s to remove most of the solution retained in the perlite. Roots and perlite were pulled out of the containers, and roots were separated from the perlite (Colpaert et al. 1999). Fine roots (those with root hairs and diameters <1 mm in nonmycorrhizal treatments or those well colonized with fungi in mycorrhizal treatments) were detached from coarse roots, mycorrhizas being included in the former fraction. Subsamples of perlite were frozen in liquid N to determine the concentration of ergosterol, a fungal biomarker. Ergosterol was analyzed via high-performance liquid chromatography (HPLC); resulting concentrations were converted to fungal biomass using conversion factors of 3.0 and 5.9 mg ergosterol/g biomass for *T. terrestris* and *S. luteus*, respectively (Colpaert et al. 1999). Dry weights of foliage, stems, and roots were determined. Dried plant and perlite material was ground with a ball mill at 200 Hz for 2 min and stored at 20–25 °C.

Leaching Experiment

To quantify the elements potentially available from the culture substrate, small aliquots (0.1 g) of unused acid-washed perlite were crushed and leached. Leaching solutions were oxalic acid, hydrochloric acid, or mixed oxalic–hydrochloric acids. The leaching experiment was conducted at three different acidity levels (pH = 2, 3, or 4) for each acid type, making a total of nine leachates. The mixed acid solution at pH 2 consisted of 0.1 mL of 1.2 M hydrochloric acid (HCl) plus 0.05 mL of 0.1 M oxalic acid, diluted to 20 mL total. The mixed acid solution at pH 3 was a further 10-fold dilution, and the pH 4 solution consisted of 0.2 mL of the pH 2 solution plus 1.0 mL of the pH 3 solution, diluted to 20 mL. Two additional treatments, employing ultrapure water as the leaching reagent, were added for a final total of 11 leaching treatments. The crushed perlite was sonicated and then leached for 2 h. Leaching solutions were then extracted using iterative centrifuging and dilution techniques. Collected leachate was then prepared for elemental analysis.

Determining Elemental Concentrations and Data Analysis

Biomass distributions between above- and belowground plant components, and between extraradical hyphae and the fungal portion of mycorrhizae, were calculated from Hobbie and Colpaert (2003). Dried, ground plant tissues (foliage + root separates) were digested in microwave

bombs with a mixed HCl–nitric acid (HNO_3)–hydrogen peroxide (H_2O_2) solution. All elemental concentrations were analyzed via atomic emission on a Varian axial inductively coupled plasma–optical emission spectrometer (ICP-OES). We then calculated the total accumulation of nutrient in foliage and roots by multiplying the measured concentrations times root or foliar biomass. These amounts were compared to the amount added during treatments plus the amount present in seedlings at the start of treatment. It is important to note that aluminum (Al) was not in the added nutrient solution but was present in the seedlings harvested prior to treatments. Element concentrations were compared in roots or foliage with a two-factor ANOVA ($\text{N} \times \text{mycorrhizal associate}$) with a Tukey post hoc test at $P = 0.05$. Additional calculated measurements were similarly compared.

RESULTS

A greater proportion of biomass was allocated belowground in low nutrient treatments than in high nutrient treatments, and this effect was independent of mycorrhizal association (Table 1). Relative allocation to mycorrhizal fungi was greater at low nutrient availability than at high nutrient availability. At high nutrient supply, allocation to fungi (in perlite and on roots) was greater for *Thelephora* than for *Suillus*, but this effect disappeared at low nutrient supply. Total biomass was about 15% more in low nutrient treatments than in high nutrient treatments and averaged 15% more in nonmycorrhizal treatments than in mycorrhizal treatments.

In Figure 1, foliar and root concentrations are plotted relative to concentrations in supplied nutrients across all treatments. Potassium and Fe were more enriched in roots, whereas Ca, Mg, and Mn were more enriched in foliage. In addition to these elements, Al was detected, although it was not included in the nutrient solution. Foliar Al averaged 50 ppm (parts per million), or 0.39% of foliar N concentration, whereas Al in roots averaged 680 ppm, or 3.1% of fine root N concentration. For normalized versus foliar and root N concentration, the relative enrichment of different nutrients in foliage versus roots ranged from 3.89 for Mn and 1.58 for Ca to 0.20 for Fe and 0.13 for Al, with Mg (1.22) and K (0.90) having values close to unity. Relative to concentrations in supplied nutrients, Mn was highly enriched in foliage, whereas Fe was highly enriched in roots.

In Figure 2, foliar nutrient concentrations are plotted against fine root nutrient concentrations by treatment. Calcium generally had similar concentrations in roots and foliage, Mn had greater concentrations in foliage than roots, and other elements had greater concentrations in roots than in foliage.

Table 1. Allocation in *Pinus sylvestris* cultures grown nonmycorrhizally or in association with either *Suillus luteus* or *Thelephora terrestris*

Treatment	Biomass total (mg)	Aboveground	Roots	Fungi on roots	Fungi not on roots	Total fungi
High nutrient						
Nonmycorrhizal	501 ± 29	0.50	0.50	—	—	—
<i>Suillus</i>	446 ± 19	0.46	0.46	0.07	0.01	0.08
<i>Thelephora</i>	456 ± 10	0.44	0.41	0.12	0.03	0.15
Low nutrient						
Nonmycorrhizal	606 ± 19	0.39	0.63	—	—	—
<i>Suillus</i>	500 ± 8	0.35	0.48	0.12	0.05	0.17
<i>Thelephora</i>	488 ± 19	0.32	0.50	0.14	0.04	0.18

Notes. Allocation is expressed as a fraction of total plant plus fungal biomass. Data were calculated from biomass values given in Hobbie and Colpaert (2003).

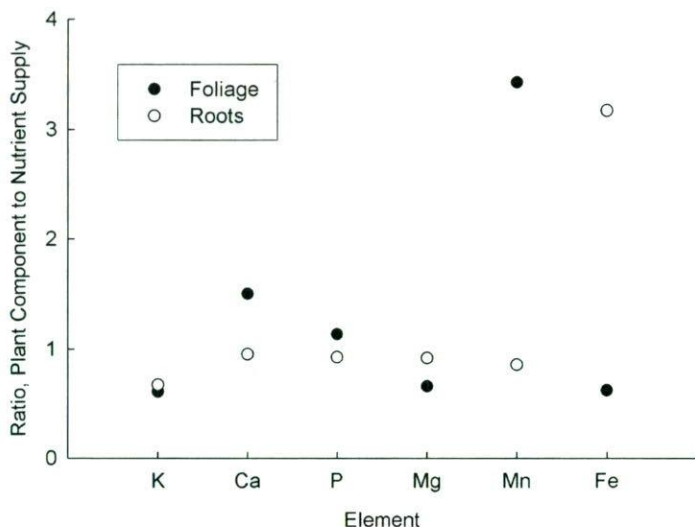


Figure 1. Across-treatment average elemental enrichment in foliage and roots. Average elemental abundances in root and foliage tissues are presented in terms of an enrichment factor compared to the elemental abundances in the nutrient supply solution.

Table 2 shows the results of ANOVA calculations of the effects of nutrient supply and mycorrhizal association on foliar and root elemental concentrations. In foliage, concentrations of Ca and Mn were greater at low nutrient supply than at high nutrient supply and for Mn were higher in *Thelephora* treatments than in other treatments ($p = 0.009$). In roots, concentrations of K and Al were greater at low nutrient supply than at high nutrient supply, whereas Mn concentrations were greater at high nutrient supply than at low nutrient supply ($p = 0.056$). Aluminum and Mg concentrations were lower in nonmycorrhizal treatments than in mycorrhizal treatments. Concentrations of K, Mg, and Mn in roots were higher in *Suillus* treatments than in nonmycorrhizal treatments.

Although equal amounts of nutrients were added in all treatments, differences in the total accumulation of nutrients by treatment or differences between the amount of nutrients added and the amount recovered in foliage and roots may indicate that other sources exist. Aluminum was not in the added nutrient solution but was present in the seedlings harvested prior to treatments. Mass balance calculations indicated that 93% more Al and 42% more Mn accumulated in foliage and roots than could be accounted for by addition of the nutrient solution and the amount initially present in the seedlings (Table 3). In contrast, foliar and root recovery of Ca was 93%, of K was 78%, and of

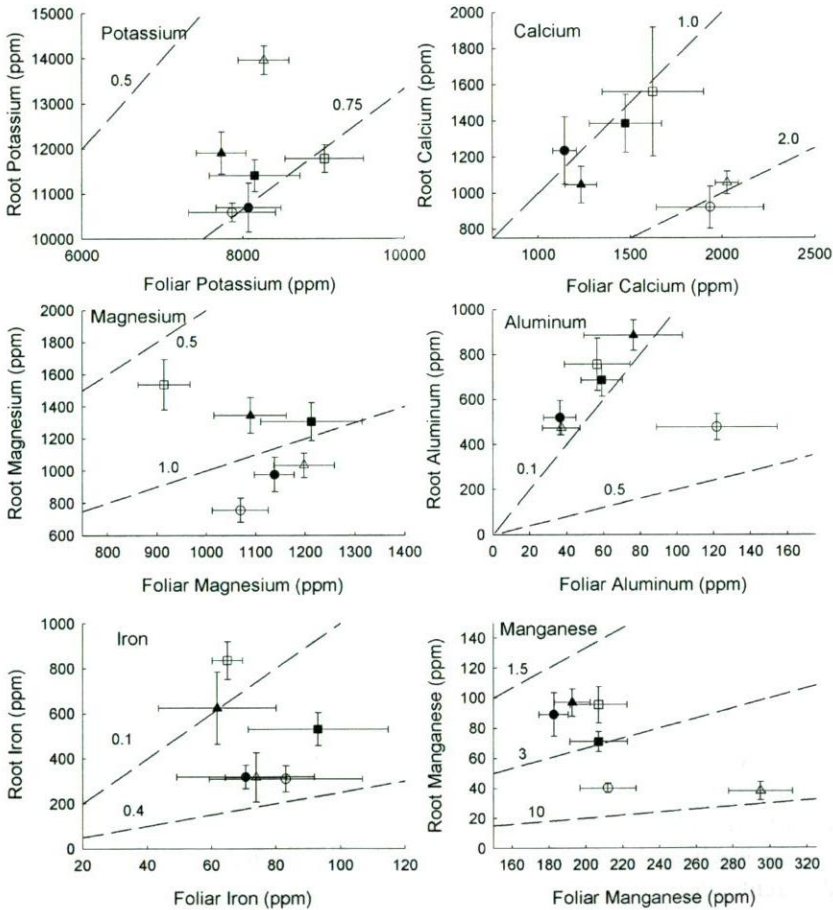


Figure 2. Cation concentrations in foliage and roots relative to nutrient supply [high nutrient, filled symbols (●■▲); low nutrient, clear symbols (○□△)] and mycorrhizal associate [nonmycorrhizal, circles (●○); *Suillus*, squares (■□); *Thelephora*, triangles (▲△)]. Concentrations are given in ppm \pm standard error ($n = 5$). Superimposed upon the plots are enrichment ratios that reflect the ratio of foliar concentrations to root concentrations.

Fe was 65% of the amount added in nutrient solution plus the initial amount present in seedlings. Total accumulation of Ca and K in roots and foliage was greater at low nutrient availability than at high nutrient availability, was greater in nonmycorrhizal treatments than in *Thelephora* treatments for Ca, and was greater in nonmycorrhizal treatments than in *Suillus* treatments for K (Table 4). Other elements did not differ significantly by treatment in total nutrient accumulation (data not shown). Because of the differing results for K and Ca for the two

Table 2. Effects of nutrient supply rate and fungal associate on elemental concentrations in foliage and roots by ANOVA

Parameter	K	Ca	Mg	Mn	Fe	Al
Roots						
Supply rate (S)	0.013	0.354	0.228	<0.001	0.961	0.056
Associate (A)	<0.001	0.328	<0.001	0.081	0.031	0.832
S × A	0.017	0.177	0.037	<0.001	0.014	0.187
Tukey–Kramer test						
Supply rate	H < L	ns	ns	L < H	ns	ns
Associate	non < Sl < Tt	ns	non < Sl, Tt	ns	non < Sl	ns
Foliage						
Supply rate (S)	0.388	0.002	0.068	<0.001	0.995	0.319
Associate (A)	0.378	0.796	0.314	0.009	0.759	0.409
S × A	0.497	0.222	0.036	0.034	0.439	0.029
Tukey–Kramer test						
Supply rate	ns	H < L	ns	H < L	ns	ns
Associate	ns	ns	ns	non, Sl < Tt	ns	ns

Notes. A post hoc Tukey–Kramer test was used at $P = 0.05$. Significant results are given in bold. Ns, not significant; H, high nutrient; L, low nutrient; non, nonmycorrhizal; Sl, *Suillus luteus*; and Tt, *Thelephora terrestris*.

mycorrhizal treatments, the Ca/K ratio was also calculated for each treatment (Table 4). The Ca/K ratio was significantly greater for *Suillus* treatments than for *Thelephora* treatments ($p = 0.050$).

In leaching experiments, elements varied in their sensitivity to the different acids used and the pH of the mixture. In general, leaching increased with lower pH. Perlite leaching experiments with oxalic acid at pH 2 yielded leachate solutions containing the greatest concentrations of cations (Figure 3). This treatment was especially effective at releasing Fe (Table 5 and Figure 3), with 20 times more Fe released at pH 2 with oxalic acid than with hydrochloric acid. In contrast, the release of Al, Ca, and K at pH 2 with oxalic acid was only about twice that released with hydrochloric acid. Perlite leached with oxalic acid released significantly more Al, Fe, Mn, Mg, and K at pH 2 than at pH 3 or 4 and released significantly more Ca at pH 2 than at pH 4. When perlite was leached with a combination of hydrochloric acid and oxalic acid, significantly more Al, Fe, Mg, Ca, and K was released at pH 2 than at pH 3 and 4, whereas Mn leaching significantly increased with decreasing pH, pH 2 > pH 3 > pH 4. Finally, leaching perlite with hydrochloric acid released significantly more Al, Fe, and K at pH 2 than at pH 3 and 4, released significantly more Mn at pH 2 and 3 than at pH 4, and released significantly more Ca and Mg at pH 2 than at pH 4.

Table 3. Total manganese and aluminum accumulated in roots plus foliage exceeded amounts in time zero (T_0) seedlings plus amount added in nutrient solution

Parameter	K	Ca	Mg	Mn	Fe	Al
Total nutrients	3452 \pm 661	503 \pm 137	391 \pm 71	54 \pm 8	86 \pm 37	118 \pm 53
Fraction of nutrients in foliage	0.48 \pm 0.09	0.61 \pm 0.13	0.56 \pm 0.09	0.80 \pm 0.07	0.19 \pm 0.09	0.12 \pm 0.08
Coeff. var.	0.19	0.21	0.16	0.09	0.49	0.68
Nutrients at T_0 plus in solutions	6170	540	862	38	131	61
Ratio, final (added plus initial)	0.78	0.93	0.45	1.30	0.65	1.93

Notes. Values are given \pm standard deviation, in μg , across all treatments ($n = 30$). Coeff. var. = standard deviation/mean.

Table 4. Total accumulation of calcium and potassium in foliage and roots of *Pinus* by treatment, with standard error, Ca/K ratios are also given

Treatment	K (μg)	Ca (μg)	Ca/K
High nutrient			
Nonmycorrhizal	3590 \pm 245	559 \pm 101	0.150 \pm 0.017
<i>Suillus</i>	2790 \pm 295	448 \pm 60	0.151 \pm 0.007
<i>Thelephora</i>	2965 \pm 250	367 \pm 33	0.125 \pm 0.010
Low nutrient			
Nonmycorrhizal	4165 \pm 180	632 \pm 24	0.153 \pm 0.019
<i>Suillus</i>	3325 \pm 110	520 \pm 20	0.157 \pm 0.009
<i>Thelephora</i>	3880 \pm 170	516 \pm 24	0.133 \pm 0.003
ANOVA			
Supply rate (S)	0.001	0.021	0.519
Associate (A)	0.004	0.021	0.038
S \times A	0.639	0.769	0.959
Tukey-Kramer test			
Supply rate	H < L	H < L	ns
Associate	<i>Suillus</i> < non	<i>Thelephora</i> < non	<i>Thelephora</i> < <i>Suillus</i>

Notes. ANOVA results at $P = 0.05$ indicate that both supply rate and associate significantly affected element accumulation, and associate significantly affected K/Ca ratios. H, high nutrient; L, low nutrient; non, nonmycorrhizal.

DISCUSSION

Nutrient Concentrations and Accumulation in Foliage and Roots

The concentration of elements in roots and foliage reflects a complex combination of factors, including the relative mobility within phloem and xylem of each element (Marschner, Kirkby, and Cakmak 1996), the sequestration of nutrients in vacuoles (Martinoia, Maeshima, and Neuhaus 2007), fixation on plant cell walls (Sattelmacher 2001), or storage in insoluble precipitates such as oxalate crystals (O'Connell, Malajczuk, and Gailitis 1983). In this study, fungal matter was an important component of root tissue in mycorrhizal treatments (about 20% of root weight on average, Table 1). Therefore, the sequestering of different elements in fungal tissue and the transfer of nutrients from fungi to plants would also greatly affect element partitioning.

Relative to root concentrations, foliage tissues were depleted in Al, Fe, and K, similar for Mg, and enriched in Mn and Ca (Figures 1 and 2). Some insight into the patterns observed in relative mobility of these elements is provided in other studies. For example, the greater mobility of Mn and Ca in xylem than in phloem can lead to foliar accumulations (Mengel and Kirkby 2001) and presumably accounts in our study for the greater concentrations of these elements in foliage than in roots

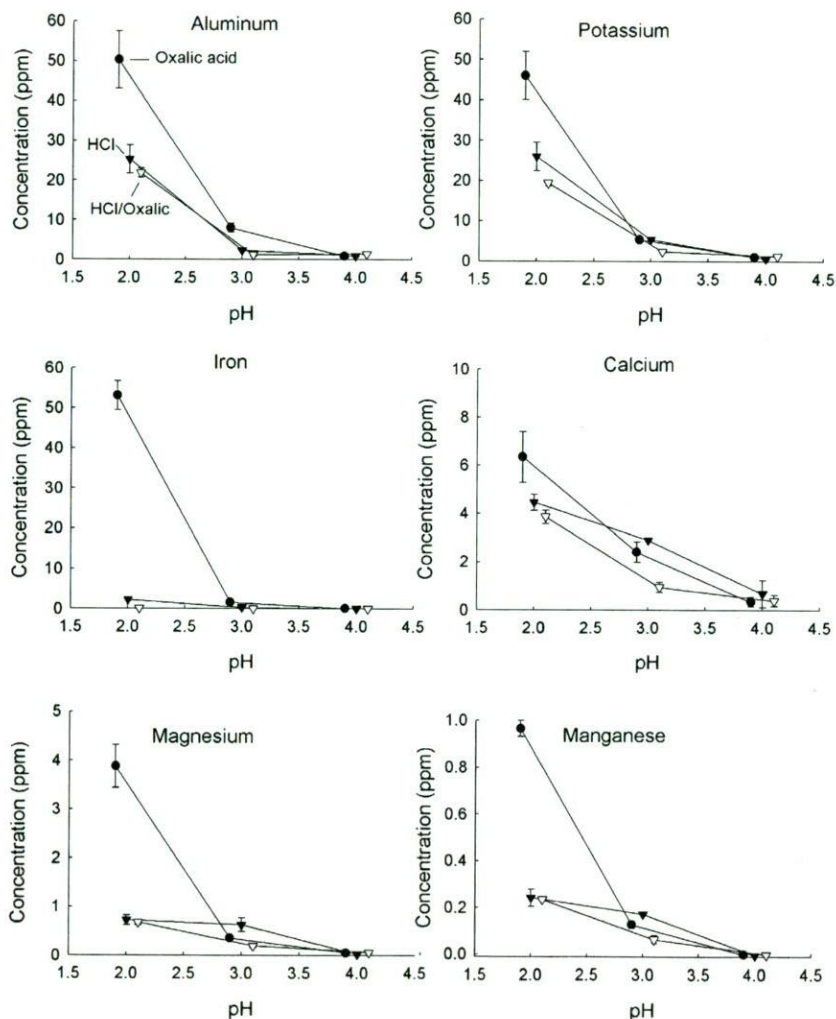


Figure 3. Cation concentrations in perlite leachate following treatment with different acids [oxalic acid, filled circles (●); hydrochloric acid, clear triangles (Δ); or oxalic plus hydrochloric acid, filled triangles (▲)] and at a pH of either 2, 3, or 4. For clarity, concentration values are slightly offset from the treatment pH for oxalic acid and oxalic plus hydrochloric acid.

(Figure 2). In another relevant study, Dambrine et al. (1995) compared nutrient fluxes in trunk xylem to total tree (*Picea abies*) uptake and reported that K fluxes were greater, Mg fluxes equivalent, and Ca fluxes lower. These patterns reflect the relative mobility of these three elements within the tree (or seedling) circulatory system, mirror the patterns of foliar to root elemental concentrations in our study ($\text{Ca} > \text{Mg} > \text{K}$), and

Table 5. Concentration of elements in perlite and concentrations in leachates from perlite leached in oxalic acid solution at pH 2

Parameter	Element					
	K	Ca	Mg	Mn	Fe	Al
Perlite digestion (ppm)	2136 \pm 53	596 \pm 17	136 \pm 17	40 \pm 1	927 \pm 59	3730 \pm 17
Perlite leachate (ppm)	23.0	3.19	1.94	0.483	26.6	25.2
Ratio, leachate: digestion	1.08 \times 10 ⁻²	5.36 \times 10 ⁻³	1.43 \times 10 ⁻²	1.20 \times 10 ⁻²	2.87 \times 10 ⁻²	6.76 \times 10 ⁻³

Note. Perlite concentrations were calculated after a microwave digestion using nitric acid, hydrochloric acid, and hydrogen peroxide.

therefore suggest that relative abundance patterns of specific elements for foliage versus roots partly reflect the relative mobility in xylem and phloem.

Additional support for the low mobility of Al and Fe causing the much lower concentration in foliage than in roots (Figure 2) is reported by Persson and Ahlström (2002). These authors found that in fine roots of *Picea abies*, concentrations of Al and Fe can be 7 to 10 times greater in mineral horizon roots than in organic horizon roots. This presumably reflects low mobility of these two elements within the plant, so that high concentrations of these elements in mineral soil relative to organic soil are reflected in their respective roots. In contrast, the high mobility within the plant of K was reflected in concentrations of K actually being somewhat less for mineral horizon roots than for organic horizon roots (Persson and Ahlström 2002).

Both nutrient supply rate and ectomycorrhizal colonization influenced nutrient distributions in our pine seedlings. Mycorrhizal colonization was greatest in low nutrient treatments. At low nutrient supply, *Suillus*-colonized roots were generally higher in Ca, Mg, Al, Fe, and Mn than nonmycorrhizal roots or *Thelephora*-colonized roots. This may reflect increased mobilization of nutrients from the perlite by *Suillus* per unit biomass, as fungal biomass was quite similar for the two mycorrhizal treatments at low nutrient availability. Because *Suillus* spp. (Danielson and Visser 1989) and *Suillus luteus* are generally found in mineral soil (Rosling et al. 2003), they may be well-adapted to extracting nutrients from minerals, whereas *Thelephora terrestris* is found in organic soil (Heinonsalo, Jorgensen, and Sen 2001; Lilleskov et al. 2002).

For Ca at low nutrient supply, *Suillus* treatments had a foliage-root concentration of about 1, whereas *Thelephora* and nonmycorrhizal treatments had a foliage-root concentration of about 2 (Figure 2). A plausible explanation for this pattern is the sequestration of Ca in the fungal portion of *Suillus-Pinus* ectomycorrhizae, because *Suillus* comprised about 25% of fine root biomass at low nutrient availability (Table 1). The crystals found in *Suillus* mantle sheaths enclosing roots (Agerer 2006) appear to contain high amounts of calcium oxalate, as is common for some ectomycorrhizal fungi (Glowa, Arocena, and Massicotte 2003).

Perlite as an Additional Nutrient Source

Based on excess accumulations of Mn and Al in seedlings relative to the supplied amounts (Table 3), nutrients must have been mobilized from perlite. The lower concentrations of Ca and K at high nutrient availability than at low nutrient availability provide additional evidence that nutrients mobilized from perlite may contribute to plant nutrient stores. Identical amounts of nutrients were added to high and low nutrient treatments. With biomass greater at low nutrient supply than at high nutrient supply, foliar nutrient concentrations should have actually declined at low nutrient relative to high nutrient supply as a result of tissue dilution. Nitrogen, the limiting nutrient in this study, declined in foliar concentrations at low nutrient relative to high nutrient supply (Hobbie and Colpaert 2003), but no other nutrient did. We conclude that an additional potential nutrient source was present in the form of the perlite.

In general, we expected greater nutrient mobilization from perlite in low nutrient treatments than in high nutrient treatments for two reasons. First, the contact time between perlite and any plant or fungal exudates that might mobilize elements from perlite was greater for low nutrient treatments (70 days) than high nutrient treatments (45 days). Second, belowground allocation and ectomycorrhizal colonization were greater in low nutrient treatments than in high nutrient treatments. These patterns would favor high production rates of organic acids in low nutrient treatments, which might enhance nutrient mobilization from perlite.

Nutrient Leaching from Perlite

In many studies, oxalate production by ectomycorrhizal fungi has been linked to nutrient mobilization, particularly of multivalent cations (Paris, Botton, and Lapeyrie 1996; Ahonen-Jonnarth et al. 2000; Cumming et al.

2001; Olssonk, Jakobsen, and Wallander 2002; Hoffland et al. 2004). We hypothesize that increased nutrient mobilization by *Suillus* compared to *Thelephora* may reflect greater production of oxalate by *Suillus luteus* than of *Thelephora terrestris*. Pine seedlings colonized by the closely related species *Suillus variegatus* produced greater concentrations of oxalate than nonmycorrhizal pines or pines colonized by *Paxillus involutus* or *Rhizopogon roseolus* (Ahonen-Jonnarth et al. 2000). Conversely, the lack of calcium oxalate crystal formation by *Thelephora terrestris* during colonization of wood ash suggests that oxalate production by *Thelephora* is quite low (Mahmood et al. 2001). These apparent differences in oxalate production may reflect functional differences in exploration strategies, with *Suillus luteus* common in mineral horizons (Rosling et al. 2003) and *Thelephora* a common pioneer genus in organic soil (Lilleskov et al. 2002). Differences in organic acid production and therefore chelation may also account for patterns of K versus Ca accumulation in *Suillus* and *Thelephora* treatments versus nonmycorrhizal treatments (Table 4), with *Suillus* treatments accumulating significantly less K than nonmycorrhizal treatments and *Thelephora* treatments accumulating significantly less Ca than nonmycorrhizal treatments. In addition, the significantly greater Ca/K ratio in *Suillus*-cultured seedlings than in *Thelephora*-cultured seedlings (Table 4) may result from enhanced mobilization of Ca by *Suillus* through oxalate production.

The argument that biogenically produced acids promote the dissolution of elements from perlite, the culture substrate, is further strengthened by examining the concentrations of elements released by perlite when leached with solutions of oxalic acid and hydrochloric acid (Figure 3). Substantial quantities of many elements, particularly divalent and trivalent cations such as Al, Ca, and Fe, were liberated by oxalic acid. In a similar experiment using tartaric acid and perlite, Al, Fe, and Mn were released in significant quantities after 4 weeks of leaching, averaging 3%, 1%, and 0.12% of the original perlite weight (Enkelmann 1992). Similarly, in our 2-h leaching experiments with oxalic acid at pH 2, about 0.2% of the original perlite weight of Al, Fe, and K were lost in leachate. Given that many ectomycorrhizal fungi secrete organic acids (Paris et al. 1995; Paris, Botton, and Lapeyrie 1996; Cumming et al. 2001; Casarin et al. 2003; van Schöll, Hoffland, and van Breemen 2006), our results indicate that mineral leaching promoted by organic acids could liberate large amounts of cations. Feldspar dissolution has also been promoted by oxalic acid (Stillings et al. 1996), and ectomycorrhizal-produced oxalate extracted Fe and Al from andesite in volcanic mineral soil (Cromack et al. 1979).

Oxalic acid was generally a more effective leaching agent than hydrochloric acid, particularly at pH 2 (Table 5, Figure 3). These results indicate that mineral weathering by fungi via organic acid secretion will

probably be most effective in microenvironments where the pH can be maintained at acidic values, such as in small mineral fissures that have restricted contact with the bulk soil solution. The pH of the bulk soil is often 4 or less in forests (Arocena and Glowa 2000; Strobel 2001), but little information exists on the pH of microsites.

The leaching results also emphasize the probable importance of strong organic chelators such as oxalate and citrate in mineral weathering. If the pH at the site of mineral dissolution can be maintained sufficiently low (\sim pH 2), then our results would suggest that leaching experiments using monovalent acids (e.g., Blum et al. 2002) may correlate poorly with leaching patterns in the field where fungi secrete large quantities of multivalent organic acids. However, if the pH is 4 or greater at the site of mineral dissolution, then leaching with monovalent acids and multivalent organic acids appears similar.

Low pH in the ectomycorrhizosphere promotes mineral breakdown and increases available concentrations of Mg, Ca, and K (Arocena and Glowa 2000). Casarin et al. (2003) reported that the pH at the surface of ectomycorrhizae of *Pinus pinaster* was 4.0–4.8 for *Rhizopogon roseolus* and 5.6–6.5 for *Hebeloma cylindrosporum*, with the bulk soil pH from 7.0 to 7.8. This study also reported that oxalate concentrations were 7–20 times greater in *Rhizopogon*-colonized roots than in *Hebeloma*-colonized roots.

In our study, the pH of the bulk solution was maintained at 4.5, but the pH of microsites at the perlite surface could have been lower. The pH of the hyphal tip can be as low as 2.5 (Gadd 1999), and van Hees et al. (2006) calculated from production rates of oxalate in ectomycorrhizal hyphae that a pH as low as 1.7 was possible in diffusion-restricted micropores. For fungi such as *Suillus* that secrete organic acids, ligand-promoted dissolution by chelators such as oxalate is much more efficient than acidolysis for liberating cations from minerals (Fomina et al. 2004).

CONCLUSIONS

Our study showed unequivocal mobilization of elements from perlite by mass balance analyses on Al and Mn in plant tissues. Mobilization from perlite for Ca and K appeared to increase as the culture period increased. Although our leaching experiments clearly showed that organic acids could release nutrients from the culture substrate, plant nutrient status was not improved by colonization by ectomycorrhizal fungi, despite strong evidence from literature that ectomycorrhizal fungi can release organic acids to mobilize nutrients. Thus, under these growth conditions where most nutrients were supplied in forms readily assimilable by plants, the nutritional benefit of ectomycorrhizal colonization was not apparent.

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